

Reprint from

RECENT ADVANCES
IN DOPING ANALYSIS
(7)

W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck-Engelke
(Editors)

Sport und Buch Strauß, Köln, 1999

D. GOUDREULT, A. FAKIRIAN:
Selegiline Urinary Metabolites In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke
(eds.) Recent advances in doping analysis (7). Sport und Buch Strauß, Köln, (1999) 61-70

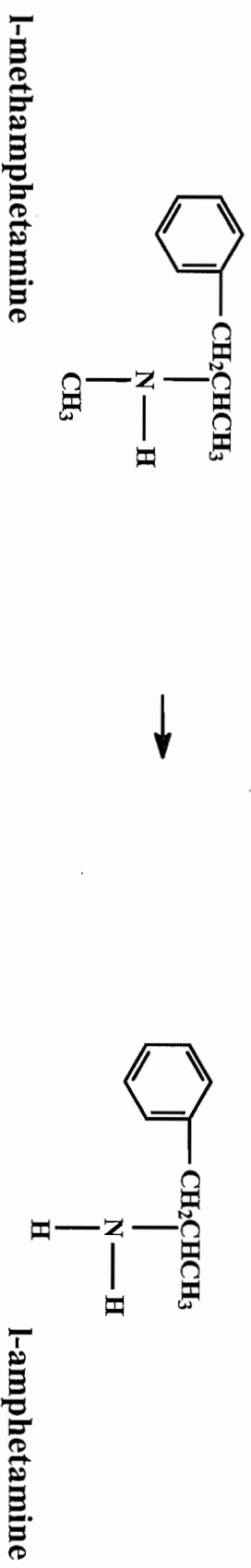
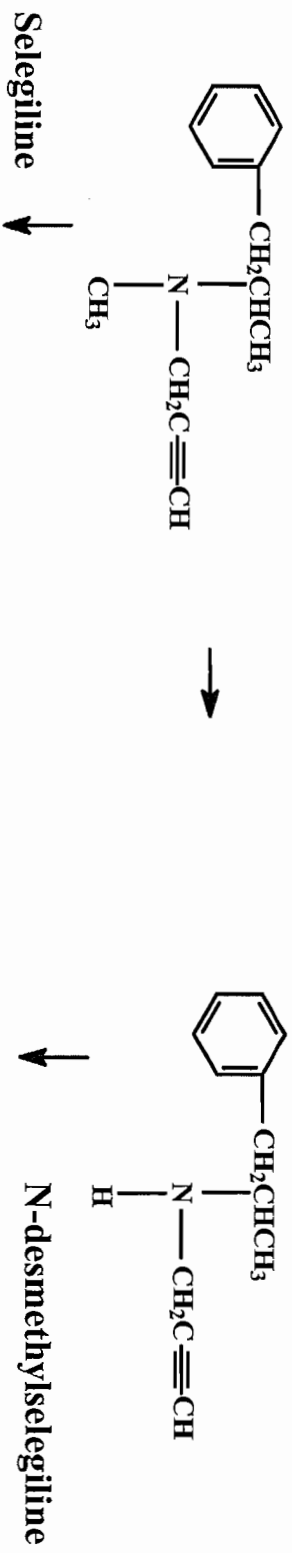
D. Goudreault , A. Fakirian

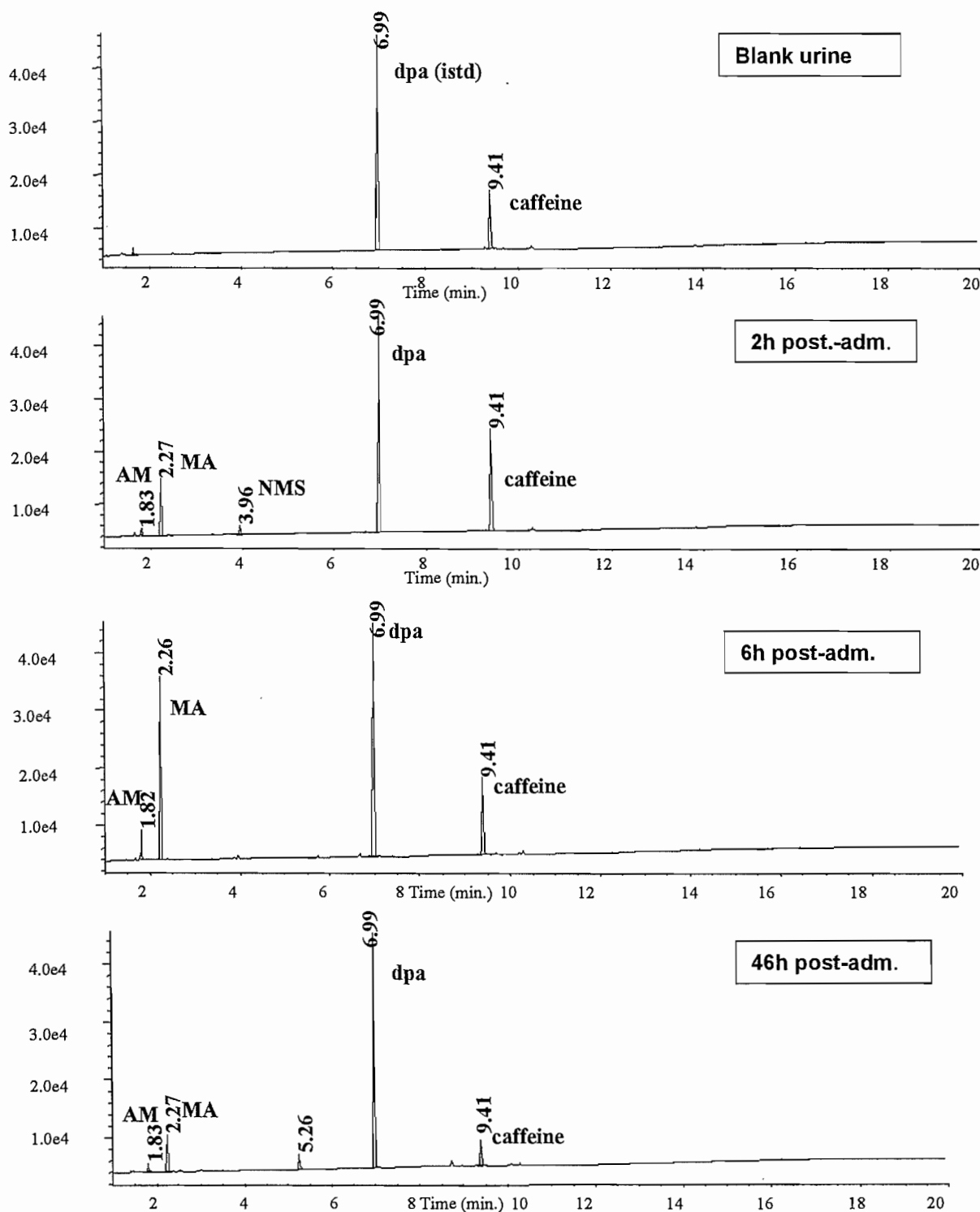
Selegiline Urinary Metabolites

INRS-Institut Armand-Frappier-Santé Humaine, Montréal, Québec

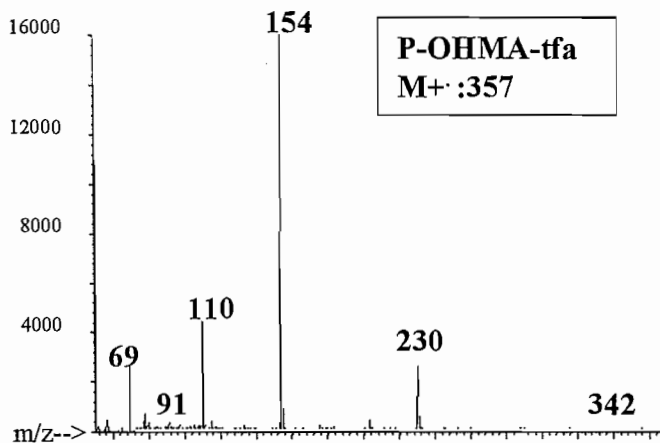
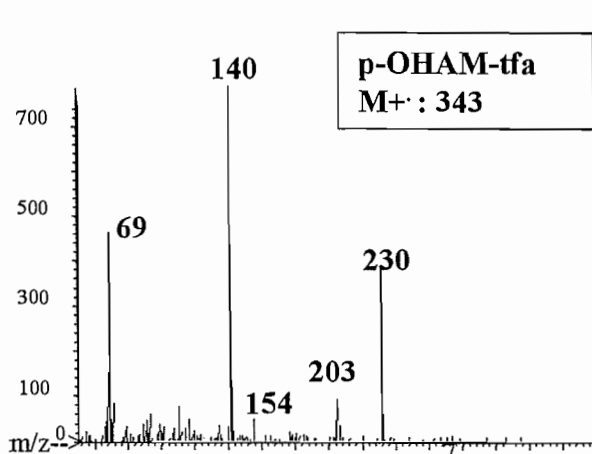
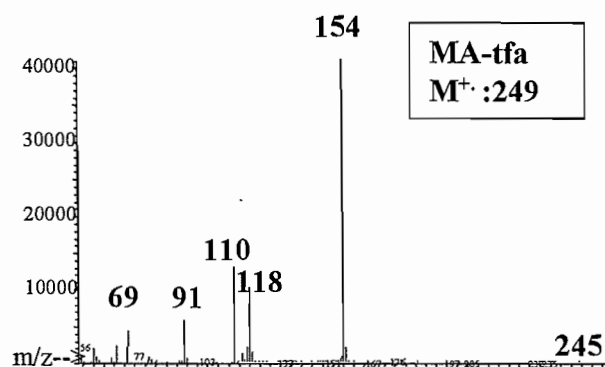
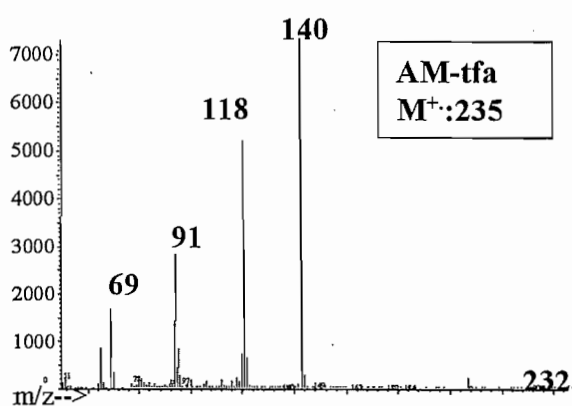
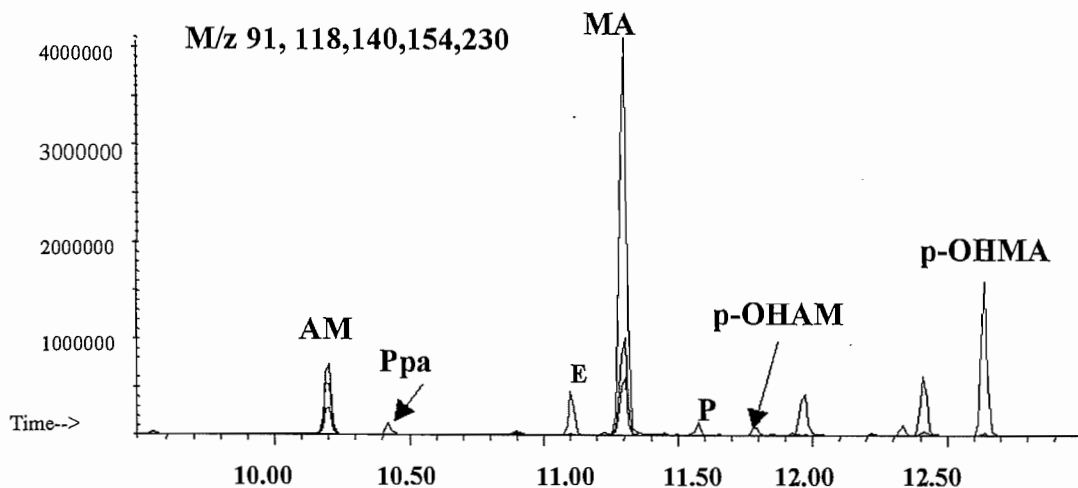
Selegiline (l-deprenyl) is a selective inhibitor of monoamine oxidase type-B (this enzyme is responsible for the deamination of benzylamine like dopamine, serotonin) and has been used in the treatment of Parkinson's disease. The use of selegiline was banned by the I.O.C. in 1998 because athletes abused it for the stimulating effects of its main metabolites being l-amphetamine (A) and l-methamphetamine (M) although the l-forms are about three to five times less active than their d-isomers. Although a few articles were recently published on the metabolism of selegiline (see figure of metabolic pathways of selegiline) we were interested to investigate it ourself to determine which compounds will be detected in our screening procedures after the oral intake of selegiline. Two female volunteers received 5mg pills called Novo-Selegiline (subject A: 10mg and subject D:15mg). Urine samples were collected for 48 hours following the administration and all urine samples were analysed in screening procedure 1 (volatile stimulants) and procedure 2 (less volatile and conjugated stimulants) by GC/NPD and the suspicious samples were analysed by GC/MSD in the full scan mode. Selegiline was detected for only 2 hours (by GC/MSD only), its N-desmethyl selegiline(NMS) for 2 to 9 hours and the amphetamines for about 2 days. To improve the detection of selegiline and NMS by GC/MSD, a solide phase extraction using Sep-Pak C₁₈™ was done prior to the liquid/liquid extraction at pH 9. The signal to noise ratio was then improved by factor two, permitting a better identification of the parent compound and NMS. Selegiline and its metabolites were excreted in the free form except for p-OH amphetamines which were partly conjugated . Chiral resolution of amphetamine and methamphetamine were performed by extractive alkylation with Mosher reagent to verify if no inversion of chirality occurred during metabolism and also to quantify the total amount of amphetamine and methamphetamine excreted. In both subjects no conversion of amphetamines occurred and the mean of l-amphetamine total excretion was 6% and of l-methamphetamine 17%. The ratio of M/A total amount was 2.3 (subject A) and 3.2(subject B) comparable to other studies and different from the intake of Vicks inhaler (containing l-methamphetamine) and d-selegiline.

Metabolic Pathways of Selegiline



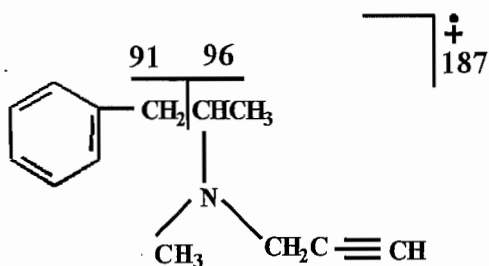
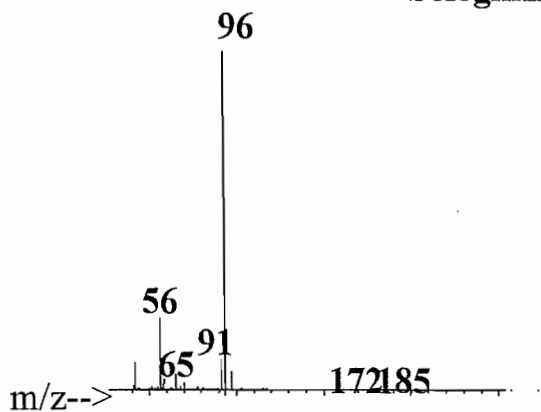


Chromatograms obtained from GC/NPD analyses of Screening procedure I from selegiline (D, 15 mg) excretion study. Dpa (diphenylamine (istd), AM(amphetamine), MA (methamphetamine), NMS (N-desmethylselegiline).

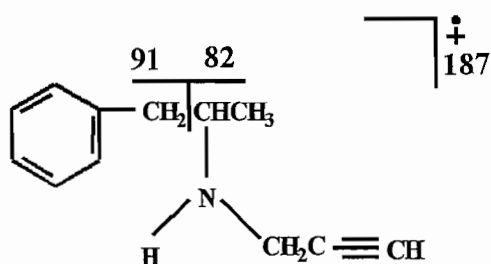
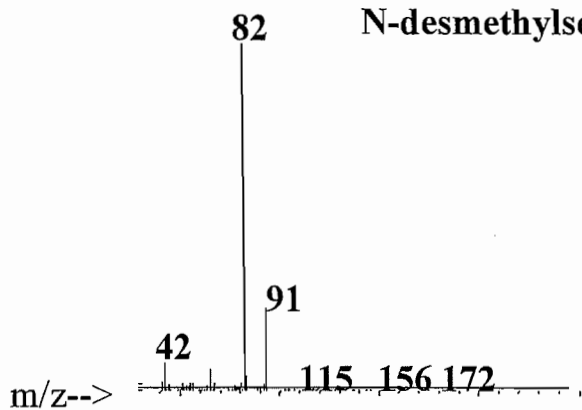


Mass spectra of selegiline metabolites as tfa derivatives obtained from GC/MS (full scan) analysis of screening procedure II (with HCl hydrolysis) from selegiline excretion study (D, 15mg) 6h after administration.

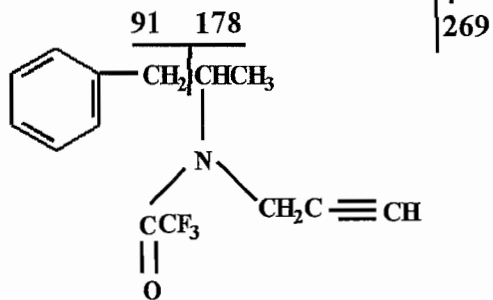
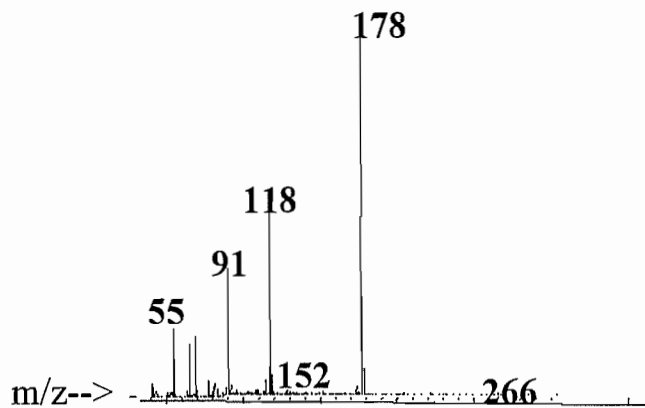
Selegiline standard



N-desmethylselegiline standard

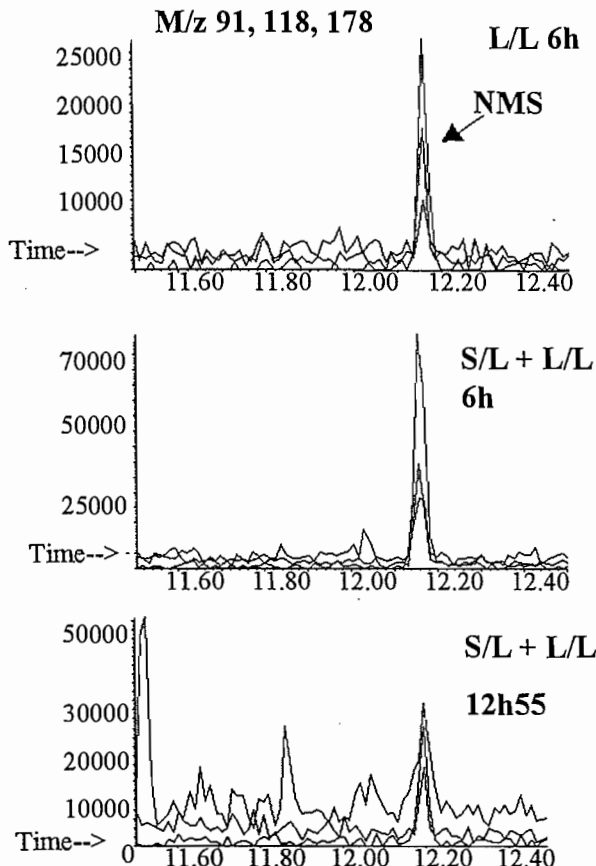


N-desmethylselegiline-tfa standard

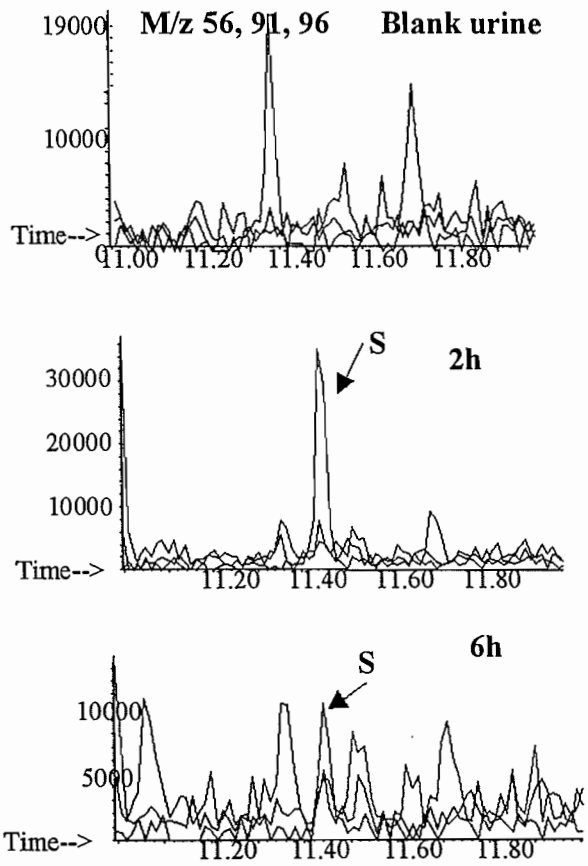


Mass spectra(full scan) of selegiline and N-desmethylselegiline authentic standards.

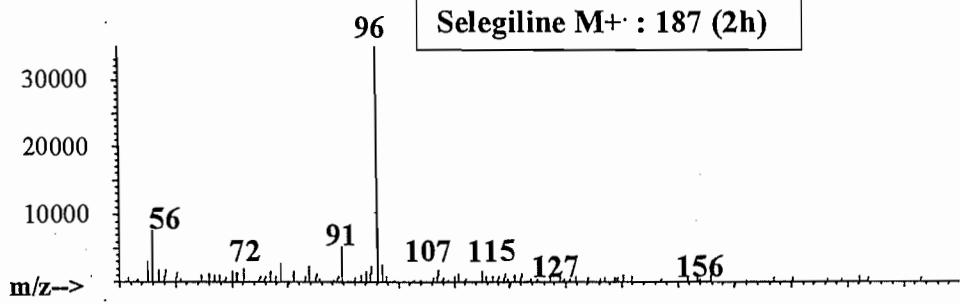
A. Comparison between (L/L) extraction (screening II, free) and (S/L + L/L) extraction (confirmation, free) of N-desmethylselegiline-tfa



B. Confirmation of selegiline by (S/L + L/L extraction, free fraction)



C. Selegiline M+ : 187 (2h)



Chromatograms and mass spectrum obtained from GC/MS (full scan) analysis of A) N-desmethylselegiline (NMS), B) and C) Selegiline (S) from screening procedure II and confirmation from selegiline excretion study (D, 15mg).

Chiral resolution of amphetamines

Extractive alkylation with Mosher reagent: (ref. R. Kazlauskas: Simple chiral derivatization, Koln Workshop 1998)

S(+)- α -methoxy- α -(trifluoromethyl)phenyl acetyl.Cl (MTPA)

To 1mL of urine

Add 5ng/ μ L of istd (l-norpseudoephedrine)

5mL of hexane

50 μ L NaOH 6M

50 μ L of 2% MTPA in hexane

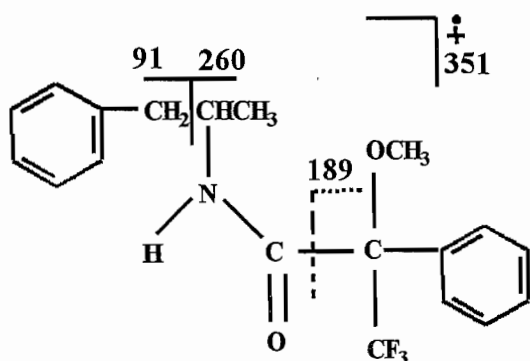
Shake 15 min. and centrifuge

Organic phase: evaporate and reconstitute in 100 μ L of ethyl acetate

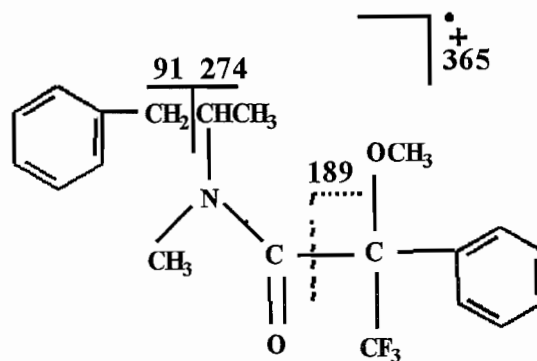
Analysis: GC/MSD

on HP5-ms column 25m in full scan and SIM (quantitation)

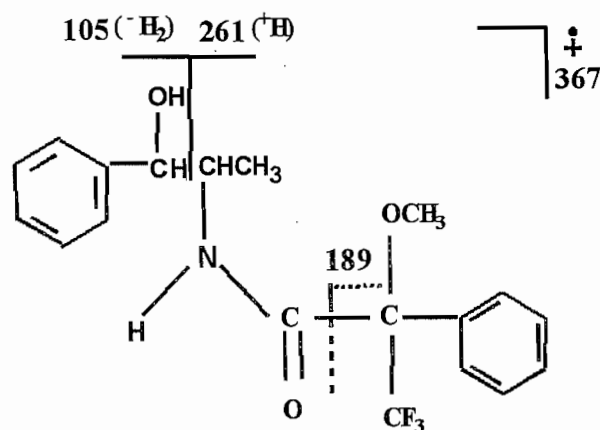
Amphetamine-MTPA

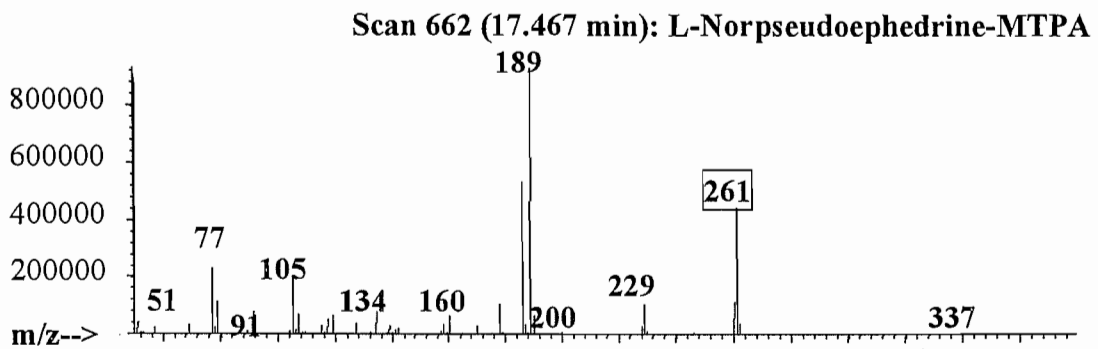
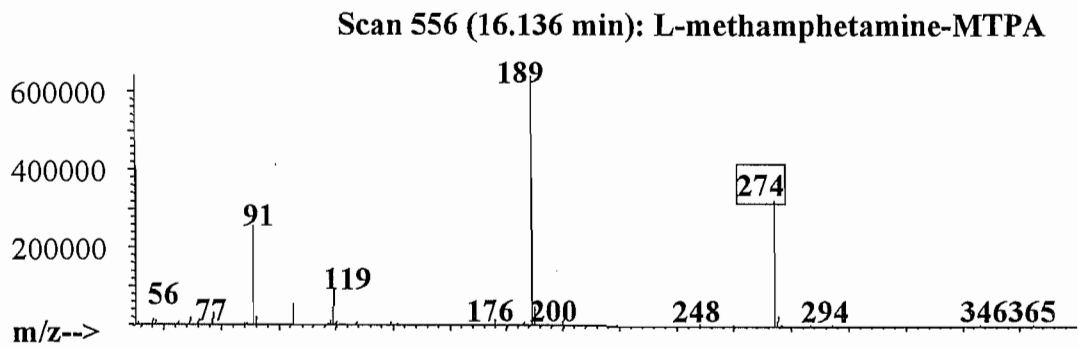
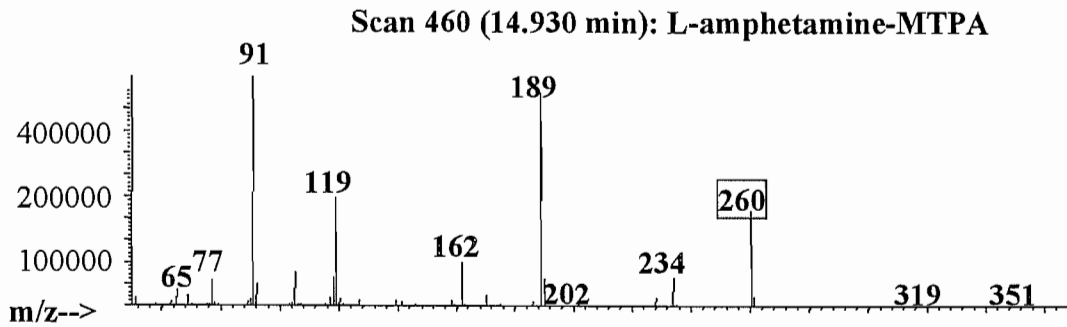
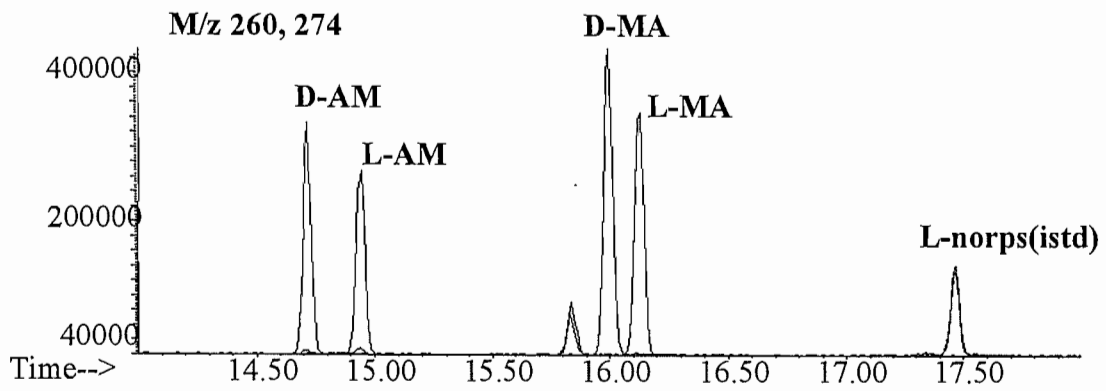


Methamphetamine-MTPA

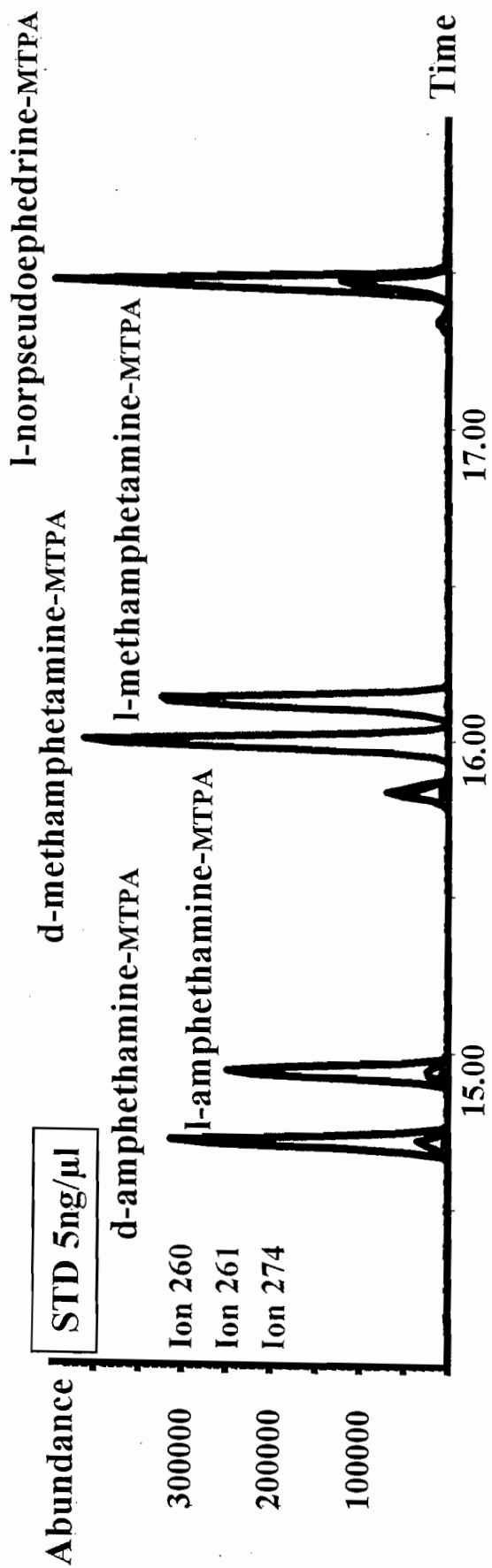
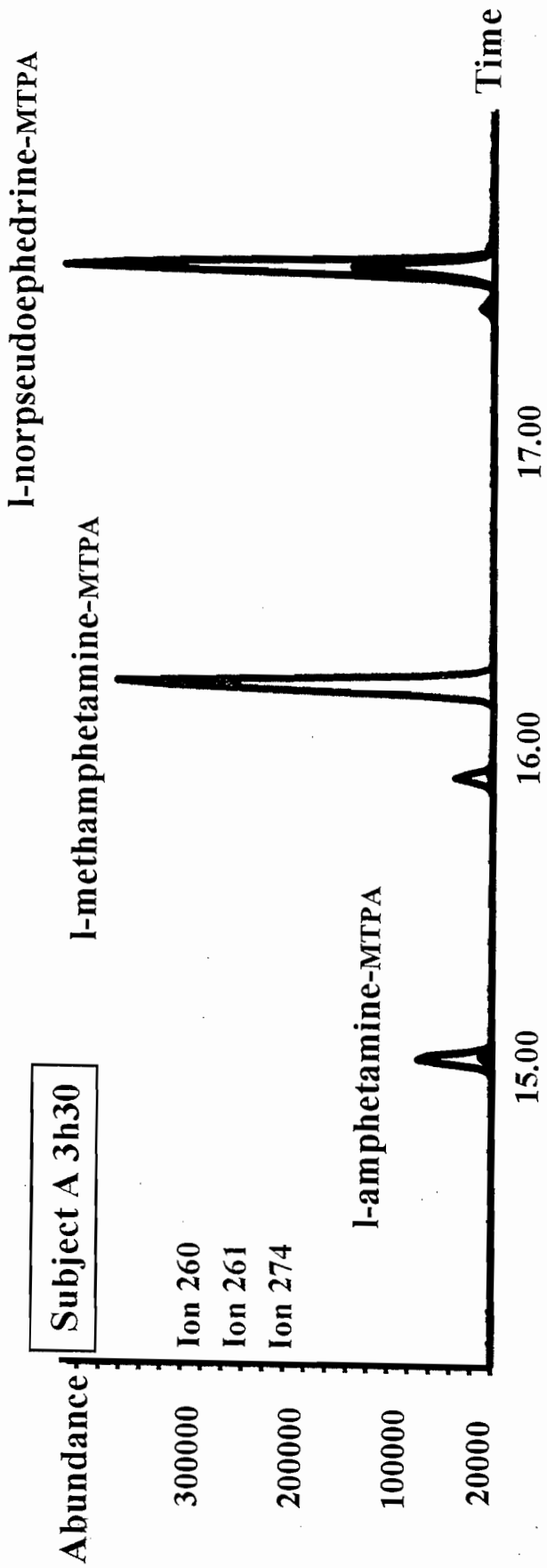


Norpseudoephedrine-MTPA





Mass spectra (full scan) of L-amphetamine, L-methamphetamine and L-norpseudoephedrine (istd) standards as their MTPA derivatives



Chromatograms of selegiline metabolites obtained from GC/MS analysis of confirmation procedure by chiral derivative MTPA. Subject A (10mg) urine at 3h30 compared to standard solution at 5ng/μl

Quantitative results of metabolites (0-48h)

Subject A (10 mg)				Subject D (15 mg)			
Time (h)	Amph. µg	Metha. µg	M/A	Time (h)	Amph. µg	Metha. µg	M/A
0	0	0	-	0	0	0	-
2	57.5	238.5	4.2	2	55.5	224.7	4
3.3	36.3	117.5	3.3	6	199.2	773.6	3.9
6	58.1	191.9	3.3	6.5	32.6	115.5	3.5
8	40.3	124.2	3.1	9.15	41.0	131.1	3.2
11	79.2	233.2	2.9	12.35	110.8	351.2	3.2
13.5	51.2	97.0	1.9	13.55	24.0	78.1	3.3
24	133.2	221.4	1.7	22.10	179.9	540.8	3.0
26	29.3	60.7	2.1	26.3	34.3	109.1	3.2
27.35	25.1	42.8	1.7	35.1	101.1	248.8	2.5
30.35	15.8	29.8	1.9	38.45	34.2	97.1	2.8
32	10.5	18.4	1.7	46	79.9	232.2	2.9
33.5	12.0	20.4	1.7				
36.5	14.8	23.0	1.6				
48	57.6	58.5	1.0				
Total Mean	6.2%	14.8%	2.3 *	5.9%	19.3%		3.2 *

*** Vicks Inhaler (pure l-methamphetamine) M/A » 8
d-selegiline M/A ~ 1 ; d-methamphetamine M/A~10**

RW Romberg et al.; J Forensic Sci 1995 Nov, 40(6):1100-2

J.L. Valentine et al.; J. of Anal.Toxicology, vol.19, Nov/Dec 1995:581-90